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IN-VITRO SUSCEPTIBILITY TO TIGECYCLINE IN MULTIDRUG RESISTANT BACTERIAL ISOLATES FROM A TERTIARY CARE HOSPITAL

Kusuma G.R.[1], Tejashree A.[2], Vijay Kumar G.S.[3]

[1]Lecturer, Department of Microbiology, BMC& RI, K.R. Road, Fort, Bangalore – 560002, Karnataka, India; [2]Professor, Department of Microbiology, JSS Medical College, Mysore – 570019, Karnataka, India; [3]Professor, Department of Microbiology, Sridevi Institute of Medical sciences and Research Institute, Tumkur 572106, Karnataka India.

ABSTRACT

Objective: The present study was conducted to evaluate the in vitro activity of tigecycline against a contemporary collection of multidrug resistant (MDR) bacterial isolates by disc diffusion and MIC by E-Test method.

Methods: A total of 100 non repetitive clinically significant MDR bacterial isolates from urine, pus, sputum endotracheal aspirates, Skin and soft tissue and surgical site infection of patients in a tertiary care teaching hospital in Karnataka, south India from March 2013 to December 2013 were included in the study. MDR bacteria tested for Tigecycline susceptibility were: Methicillin resistant *S.aureus* (MRSA) (15), ESBL producing Escherichia coli (*E.col)* (15), *Klebsiella pneumoniae* (35) and MDR *Acinetobacter species (35).*

Result: Tigecycline was found to be effective against all MRSA, MDR E.coli and Acinetobacter isolates by disc diffusion and E-test method. Among the selected *K. pneumoniae* isolates all were sensitive by disc diffusion and 34 (97.1%) were found sensitive with the MIC range 0.25- 1.5µg/ml, One (2.9%) isolate was found intermediate resistant with the MIC of 3µg/ml by E-test.

Conclusion: To conclude, the present study showed that, tigecycline is a potent antimicrobial agent against MRSA, ESBL producing *Enterobacteriaceae Acinetobacter species and* disc diffusion is simple to perform, highly reproducible and inexpensive method to predict tigecycline resistance. It is also prudent to reserve tigecycline for life threatening infections.

Key Words: Tigecycline, MDR, E-Test, India

INTRODUCTION

Antimicrobial resistance has been identified as one of the major challenges facing public health, as antimicrobial resistance has increased rates of morbidity, mortality and socioeconomic costs[1].

The rates of multiple drug resistance are increasing among Enterobacteriaceae, Gram-positive pathogens such as methicillin resistant *Staphylococcus aureus* [MRSA] vancomycin resistant enterococci [VRE] and MBL producing non fermenting gram negative bacilli that commonly cause serious life-threatening diseases and presents a difficult challenge for clinicians by limiting the armamentarium of potentially active antimicrobial agents. This rapid rate of microbial evolution underscores the urgent need for the development of new agents that overcome existing mechanisms of resistance displayed by multidrug-resistant bacteria[2]

In this scenario, research to find tetracycline analogues, that circumvented the resistance mechanisms, has led to the development of a novel group of drugs called glycylcyclines, the most promising compound being the 9-tertbutyl glycyclamido derivative of minocycline-tigecycline[3]

Tigecycline [TGC] is the first of a new class of modified tetracycline antimicrobials known as glycylcyclines. It exerts its bacterostatic effect by binding to a single high affinity intracellular site of the bacterial 30S ribosome and inhibits protein biosynthesis as with other tetracycline derivatives, but its unique feature is its ability to evade the major determinants of tetracycline resistance that provide ribosomal

Corresponding Author:
Kusuma G.R., Lecturer, Department of Microbiology, BMC & RI, K.R. Road, Fort, Bangalore – 560002, Karnataka, India.
Mobile: 9448120655; E-mail: kusuma.bmc@gmail.com

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protection. Its structural modification is the addition of a 9-*t*-butyl-glycylamido side chain to the central skeleton of minocycline. This provides the drug with an expanded spectrum of activities, including those against susceptible and multidrug-resistant Gram-positive and Gram-negative organisms, anaerobes, and atypical Mycobacteria but tigecycline has poor activities against certain organisms, most importantly *Pseudomonas* spp. and *Proteus* spp. as they carry inherently encoded resistance-nodulation-division (RND) efflux pumps that confer decreased sensitivity. [5&4]

Tigecycline was approved in 2005 by the U.S. Food and Drug Administration (FDA) and in 2006 by the European Medicines Agency for the treatment of complicated skin and skin structure infections and complicated intra abdominal infections [6]

Thus the present study was conducted to evaluate the in vitro activity of tigecycline against a contemporary collection of multiple drug resistant bacterial isolates from clinical specimens by disc diffusion and MIC by E-Test method.

MATERIALS AND METHODS

This prospective study was conducted in the microbiology department, at JSS hospital, Mysore a tertiary care teaching hospital in Karnataka, South India from March 2013 to December 2013.

A total of 100 non repetitive clinically significant MDR bacterial isolates were included in the study. Susceptible bacteria, Pseudomonas *spp*. *and Proteae* were excluded from the study as tigecycline has decreased activity against *Pseudomonas* and *Proteae.*

The specimens such as urine, pus, sputum and endotracheal aspirates, swabs from Skin and soft tissue and surgical site infection inection received for culture and sensitivity testing from various departments in our hospital were immediately processed and initial isolation, identification and antibiotic susceptibility testing was done according to standard recommended procedure in the clinical laboratory.[7]

All Gram negative pathogens were tested for ESBL production by CLSI phenotypic confirmatory method using ceftazidime (CAZ) and ceftazidime +clavulinic acid (CAZ+clav) disc according to CLSI guidelines *Acinetobacter* species were tested for MBL production by imipenem-EDTA combined disk test11. Screening of MRSA was done by using cefoxitin (30µg) disc. Interpretation was done according to clinical laboratory standards institute [CLSI] guidelines[8].

MDR Gram negative bacterial isolates and MRSA randomly selected for tigecycline susceptibility testing by disc diffusion & E –test were MRSA (15), ESBL producing Escherichia coli[*E.coli*] (15), *Klebsiella pneumoniae* (35) and MDR *Acinetobacter species (35),*both MBL and non MBL producers were included.

Selected isolates were tested for tigecycline susceptibility by Kirby Bauer disc diffusion method using commercially available tigecycline (15µg) disc. [Hi media]. *S.aureus* strain 25923 and ATCC *E.coli* strain 25922 were used as control.7&8.

The minimum inhibitory concentration of tigecycline for all the selected MDR bacteria was done by E- Test method. The E-test strips were procured from Biomeriux SA, France, and the test was performed according to manufacturer's instructions. Since there were no CLSI recommended interpretative criteria for MIC of tigecycline, the US FDA breakpoints were used. For Enterobacteriaceae Sensitive isolates were those with MIC ≤ 2µg/ml, and ≥ 19 mm zone size and resistance was defined as MIC ≥ 8 µg/ml and zone size ≤ 14 mm. For *S. aureus* MIC ≤ 0.5µg/ml and ≥ 19mm zone size was considered sensitive [9]

http://www.iomcworld.com/ijcrimph/files/v05-n08-02.pdf

Since there were no CLSI recommended interpretative criteria for tigecycline, the US FDAbreakpoints:

RESULTS

All randomly selected MRSA [15] isolates for tigecycline susceptibility testing by disc diffusion & E –test were found susceptible to tigecycline by both methods. The MIC range by E-test was 0.125 – 0.032µg/ml. The range of diameter of the zone of inhibition around tigecycline disc was 19mm -25mm. There was a gradual reduction of tigecycline zone diameter as the MICs for the isolates increased.

All selected *E.coli [15]* and *K.pneumoniae* [35] isolates were ESBL producers and resistant to Amikacin, Gentamycin, Cephalosporins, Ciprofloxacin, Cotrimoxozole. Among K.pneumoniae isolates 30 were resistant to Piperacillin/tazobactam and 06 isolates were resistant to Imipenem.

Susceptibility to tigecycline among *E.coli* isolates was found 100% by both methods. MIC range by E-test was 0.064 – 0.25µg/ml. The diameter of the zone of inhibition around tigecycline disc was in the range of 21mm – 25mm and there was correlation between MIC and disc diffusion.

Among the selected *K.pneumoniae* isolates, 34(97.1%) were found sensitive by E-test with the MIC range 0.25- 1.5µg/ml. One isolate was found intermediate resistant with the MIC of 3µg/ml . All isolates were in the sensitive range 19mm – 21mm by disc diffusion.

*Acinetobacte*r species [35] were resistant to Amika-

cin, Gentamycin, Tobramycin, Cefipime, Piperacillin/ tazobactam and Ciprofloxacin. 11 isolates were resistant to Imipenem.

By disc diffusion method all *Acinetobacter*species were in the sensitive range. The zone of inhibition remains between 19mm-24mm. All isolates were found to be sensitive with the MIC range between 1.5 – 0.032 µg/ml by E-test.

DISCUSSION

Tigecycline shows high potency against Gram-negative bacilli belonging to family Enterobacteriaceae in whom multi-drug resistant strains have emerged as important nosocomial pathogens. Tigecycline is also very active against non-fermentative GNB, such as *Acinetobacter spp*. and tigecycline has promising microbiological, pharmacodynamics & pharmacokinetic profile. Therefore it is considered as a good alternative to treat infections due to multidrug resistant organisms.[10]

In our study tigecycline showed 100% activity against all, MRSA by a concentration of ≤0.25 µg/ml of tigecycline with a zone of inhibition of ≥ 19mm. The results of our study were in concordance with study conducted by Bijayani Behra et al[11], Rouchelle Tellis et al[6], Manisha mane et al[13], Maria souli et al[12]

ESBLs are one of the most evolving mechanism of antibiotic resistance among the family *Enterobacteriaceae*. and therapeutic options are limited. Although Carbapenems are the drug of choice in the treatment of infections due to ESBL producing strains of Enterobacteriaceae, the emergence and proliferation of bacteria resistant to this important group of drug is jeopardizing the use of carbapenems and options are limited to tigecycline, colistin and polymyxin [13&14] .

In our study tigecycline remains an important option against the management of *E.coli* infections by retaining 100% *in vitro* activity against *E.coli* isolates with the MIC_{90}≤0.25. µg/ml by E-test. All isolates showed zone of inhibition ≥ 19mm. the zone diameter remains within a range of 21mm- 25mm. and shows the correlation between E-test and disc diffusion. Similar results were found in studies conducted by Soham Gupta et al, Shanti. M et al[15], Anand Manoharan et al[16], Rouchelle Tellis et al, Te-Din Huang et al[17],Goran Kronvall et al[18] .

Among our selected *K. pneumoniae* isolates 34/35 (97.1%) were found sensitive with the MIC range 0.25- 1.5µg/ml. One isolate was found intermediate with the MIC of 3µg/ml by E-test. None were found resistant /intermediate by disc diffusion. All the isolates showed zone of inhibition ≥ 19mm. the zone diameter remains within a narrow range of 19mm-21mm.Other studies by Shanthi.M et al 100%, Anand Manoharan et al,100% Simit Kumar et al[19] 100% BijayiniBehera et al 97% Soham Gupta et al[20] 85.7% also showed high in vitro activity of tigecycline against *K.pneumoniae*. In the study conducted by Subhash C. Arya[21]66% were found sensitive. It was also noted that in our study, increased MIC of 1-3µg/ml in *K.pneumoniae* compared to *E.coli* with MIC_{90}≤ 0.25µg/ml. Similar results with increased MIC were found by Soham Gupta et al, and Maria Souli et al.

All selected *Acinetobacter species* isolates were in the sensitive range The zone of inhibition between 19mm-24mm. The MIC remains between 0.75 – 0.032 µg/ml with MIC_{90} ≤0.75 by E-test. As there was no break points for MIC and disc diffusion for *Acinetobacter*,cut off values given for Enterobacteriaceae was used by other studies which we also has followed in our study. It was observed that there was gradual reduction in the size of zone of inhibition as MIC increases. This was also observed by Bijayini Behera et al. In our study, the E test correlated 100 percent with the inhibition zone diameters, which was in contrast to the findings of a study which was done by Behera et al. But, it was similar to the findings of a study which was done by Venezia et al[22], SurapeeTiengrim A et al[23].

In our study Tigecycline remains as a good option for the management of MRSA, MDR *E.coli, Klebsiellssps.* and *Acinetobacter* infections by retaining 100% activity against these pathogens .

We carried out the in-vitro activity of tigecycline by disc diffusion and E test. By both methods all isolates were found to be sensitive except one *Klebsiella* isolate which was found to be intermediate by E-test and sensitive by disc diffusion. Thus we found no significant difference between these 2 methods. Disc diffusion method is simple to perform, highly reproducible and inexpensive while E test is though costly for routine use, it can be used to determine MIC.

Tigecycline does not require dose adjustment in patients with impaired renal function and is conveniently administered every 12 h. It has a long terminal half-life and a large volume of distribution and has low potential for organ toxicity and drug-drug interactions these properties, make the use of this antibiotic relatively uncomplicated, but patients may develop moderate-to-severe nausea and vomiting during tigecycline therapy. It can be used as a life saving antimicrobial in polymicrobial infections due to Gram-positive and enteric Gram-negative bacteria.

To conclude, the present study shows that, tigecycline is a potent antimicrobial agent against MRSA, ESBL producing *Enterobacteriaceae* and multi-drug resistant *Acinetobacter baumannii and* disc diffusion is simple to perform, highly reproducible and inexpensive method to predict tigecycline

resistance. However, use of tigecycline needs to be strictly monitored to prevent development and dissemination of resistance against this one of the last available antimicrobial molecule and it is prudent to reserve tigecycline for life threatening infections.

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Table 1: Showing ranges of the zone of inhibition by disc diffusion and MIC by E-test

Isolates	Zone of inhibition	MIC
MRSA	21 mm–25 mm	0.032-0.125µg/ml
E. coli	21 mm–25 mm	0.064-0.25µg/ml
Acinetobacter species	19 mm–24 mm	0.032-1.5µg/ml
Klebsiella pneumoniae	19 mm–21 mm	0.25-1.5µg/ml **One isolate – 3µg/ml**

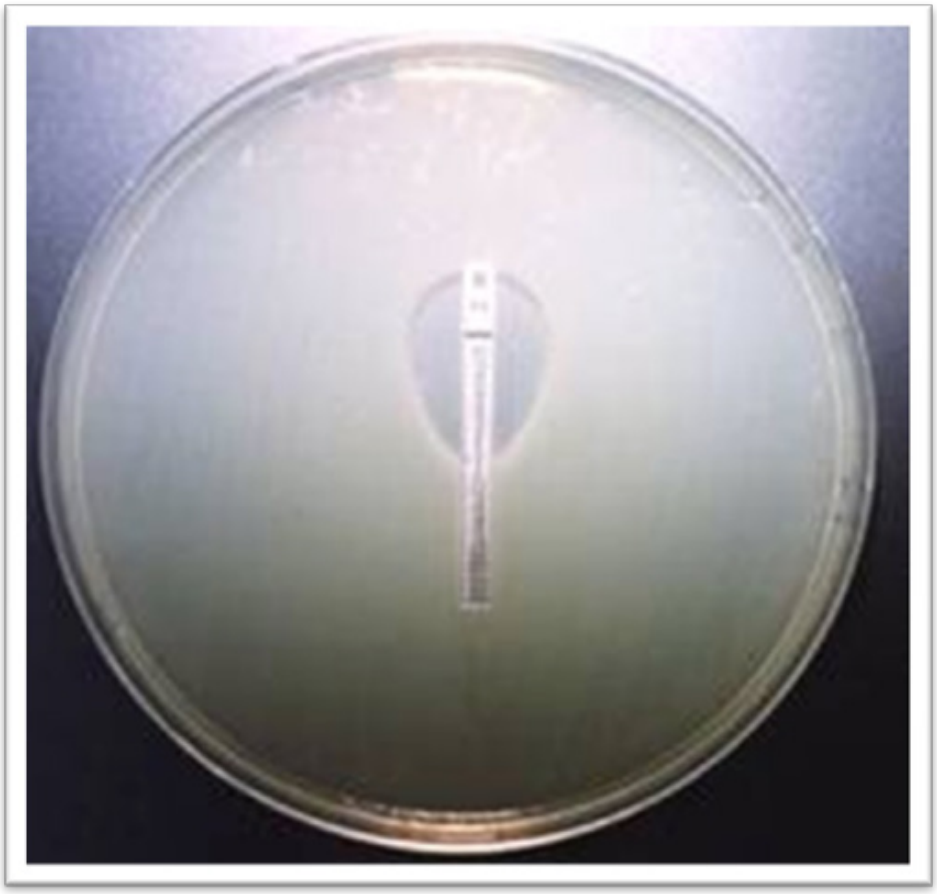

Figure 1: Muller Hinton agar plate with E-test strip showing MIC of 3µg/ml.